

THE INFLUENCE OF DEUTERIUM DEPLETED WATER ON THE HEMATOCRIT AND THE LEUKOCYTE FORMULA IN RATS INTOXICATED BY CADMIUM CHLORIDE

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Abstract: The present study shows the influence of the deuterium-depleted water (DDW) as compared to the drinking water on the haematocrit and the leukocyte formula on the cadmium chloride intoxication in rats divided into 6 batches comprising 10 individuals each. The blood samples were collected directly from the heart (intracardiac puncture) under narcosis; in accordance with the animals' protection law No. 475/2006. The obtained data have been processed statistically; calculating the mean values and the main variability factors ($\bar{x}$; S_x ; s ; C_v) that have been analysed afterwards with the help of the Mann-Whitney (Wilcoxon) test. From both the healing and preventing effect point of view as a result of the DDW administration an improvement of the non-specific defence capacity of the organism was produced. This can be a starting point for further studies that can establish the DDW status as adjuvant in the prevention and /or treatment of the different pathological states.

INTRODUCTION

Water is one of the main compounds of the living matter and takes part in all fundamental processes of the organism. The massive pollution of the last decades affected also the water both the surface and the ground water; cadmium being one of the main factors. It is known that cadmium toxicity affects the liver; kidneys; the blood; the heart; and the digestive apparatus. At the haematic level cadmium is mainly bound to plasmatic proteins and the erythrocytes; producing the Ca^{2+} ATP+ase pump inhibition with calcium accumulation in the erythrocyte up to a lethal level (14); or it causes both anaemia and the altering of the antioxidant status at the blood level (8;9) One of the reasons of the anaemia effect can be that the administration of cadmium chloride rises the blood level of glucosis urea creatinine and bilirubin (4)

The natural water is a mixture of H_2O and D_2O . The ratio between the number of hydrogen and deuterium atoms ($R = H/D$) is about 150 ppm (parts per million). Light water also called deuterium-depleted water (DDW) has an isotopic ratio R smaller than 80ppm. The deuterium concentration increase leads to heavy water (D_2O) that has negative biological effects (13; 16). The DDW has positive effects upon the leukocytes (specific and non specific immunostimulation) and the erythrocytes (2;6;7;8;10).

The present study wants to show the influence of DDW upon some sanguine parameters; i.e. the haematocrit and the leukocyte formula at the cadmium chloride intoxication in rats.

MATERIAL AND METHODS

The experiment was carried out on 60 adult Wistar rats with a body weight between 220-240 g that were fed with dry food (a cereal mixture: 40% maize; 40% wheat; 10% sunflower) and water. The rats were divided into 6 batches; each comprising 10 individuals; as follow:

- Batch 1 (1DW) – the control one – got drinking water (DW) ad libitum for 61 days.
- Batch 2 (2DW) - got DW for 30 days and on day 31 they were administrated cadmium 20 ppm/kg under the form of CdCl_2 . The animals were slaughtered 24 hours after the cadmium administration.
- Batch 3 (3DW) – got DW ad libitum (deuterium content 30 ppm/l) for 30 days and on day 31 they cadmium 20ppm/kg in form of CdCl_2 was orally administrated. The rats got drinking water ad libitum for 30 days more and thereafter they were slaughtered.
- Batch 4 (1DDW) - got DDW ad libitum (deuterium content 30ppm/l) for 61 days and the rats were slaughtered thereafter.
- Batch 5 (2DDW) – got DDW ad libitum (deuterium content 30ppm/l) for 30 days and on the day 31 they were administrated cadmium 20 ppm/kg under the form of CdCl_2 . The animals were slaughtered 24 hours after the cadmium administration.
- Batch 6(3DDW) - got DDW ad libitum (deuterium content 30 ppm/l) for 30 days and on day 31 they got cadmium 20ppm/kg in form of CdCl_2 . The rats got DDW ad libitum for 30 days more and thereafter they were slaughtered (table 1).

Table 1

Work protocol for the deuterium-depleted water and cadmium chloride experiment in rats

Batch	Time interval				
	1-30 days	day 31	day 32	32-61 days	day 62
1DW	Drinking water				slaughtering
2DW	Drinking water	CdCl ₂ (20 ppm /kg)	Slaughtering		
3DW	Drinking water	CdCl ₂ (20 ppm /kg)	Drinking water		slaughtering
1DDW	DDW				slaughtering
2DDW	Deuterium-depleted water	CdCl ₂ (20 ppm /kg)	Slaughtering		
3DDW	Deuterium-depleted water	CdCl ₂ (20 ppm /kg)	Deuterium-depleted water		slaughtering

The DDW has been obtained from the heavy water factory ROMAG Turnu Severin. The blood samples were collected directly from the heart (intracardiac puncture) under narcosis; in accordance with the animals' protection law No. 475/2006 (17). The haematocrit (Ht) and the leukocyte formula (LF) have been determined (19).

The obtained data have been processed statistically; calculating the mean values and the main variability factors ($\bar{x}$; S_x ; s ; C_v) that have been analysed afterwards with the help of the Mann-Whitney (Wilcoxon) test.

RESULTS AND DISCUSSIONS

The values obtained for the haematocrit are rendered in table 2.

As for the batches treated with DW; the mean value of the haematocrit increases significantly ($p=0.0002$) from 44% (DW1) to 48% (DW2) after administrating CdCl_2 . This happens probably because of the rapid mobilisation of the red cells of the reservoir organs. In batch DW3 a significant decrease of the mean value of the haematocrit to 37.66% (fig.4) can

be observed. The haematocrit decrease under the control value 44% (1DW) can be the result of the haematotoxic effect of the cadmium (4;8;9).

Table 2
The statistical parameters of the haematocrit in rats treated with drinking water and deuterium-depleted water in CdCl₂ (20 ppm /kg) intoxication conditions

	1 DW	2 DW	3 DW	1 DDW	2DDW	3 DDW
x	44.00	48.00	37.66	40.50	40.50	41.00
Sx	0.39	0.45	0.50	0.47	0.58	0.40
s	1.25	1.43	1.58	1.49	1.84	1.27
Cv	2.83	2.99	4.18	3.68	4.55	3.10

x-mean; Sx- average mean error; s- standard deviation; Cv – variability coefficient

compared with DW but it is also possible that the DDW would have improved the oxygen-carrying capacity of the red cells and their reducing. This would be a starting point for new researches on the DDW role in the modification of the oxygen-carrying capacity of the blood.

The obtained values of the leukocyte formula (LF) are shown in table 3.

The values obtained for the leukocyte formula at batch 1DW are within the normal limits and in accordance with the specialized literature (1; 3). An exception makes the monocytes percentage that is slightly increased. At the batch 2DW the neutrophils percentage increases (41%) as compared with the batch 1DW (27.75%) and the percentage of all the other categories of leukocytes decreases. The values of the mean percentages of the neutrophils and lymphocytes are at the batch 3DW almost the same as those of the control batch (with insignificant differences as compared with 1DW; $p=0.3258$; and $p=0.8703$ respectively). The same can be observed and at the monocytes; but with significant difference ($p=0.0002$). A significant increase ($p=0.0002$) of the mean eosophiles percentage can also be observed. The modifications observed reveal the fact that the organism has started defending phenomena comprising three phases: neutrophilic fight; monocytary fight; and healing (5; 12) consecutive to cadmium chloride administration.

At batch 2DDW in comparison with batch 1DDW an increase of the neutrophils and monocytes (40.5% and 7.30% respectively) and a decrease of the eosinophiles (1.45%) and lymphocytes (54.00%) can be observed. At batch 3DDW the increase of the neutrophils (48.10%) and eosinophiles (4.45%) is associated with the decrease of the lymphocytes (40.90%) and the monocytes (6.55%).

The observed variations are statistically significant ($p=0.0002-0.0138$) with the exception of the lymphocytes percentage decrease ($p=0.4497$) at one day and that of the monocytes ($p=0.0696$) at 31 days after the cadmium chloride administration. It can be said that the DDW amplifies the neutrophilic reaction phase by stimulating the participation of the

The haematocrit variation within the DDW batch was insignificant ($p=0.7337$; $p=9698$; $p=0.5967$).

The haematocrit of the DDW batches (fig. 1) was in comparison with that of the DW batches lower with significant differences ($p=0.0004$; $p=0.0002$; $p=0.0015$). This means that the DDW had a better protection against the haematotoxic effect of cadmium

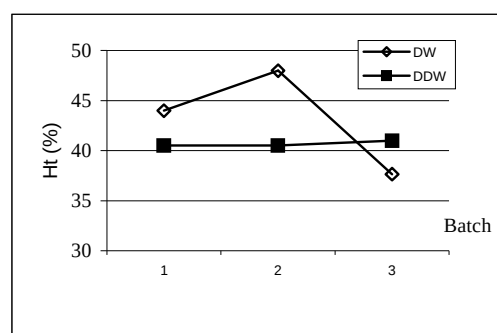


Fig. 1 Comparative mean values of the haematocrit in rats treated with drinking water (DW) and deuterium-depleted water (DDW); intoxicated with cadmium chloride

monocytes at one day after the intoxication and by prolonging the neutrophils activity up to the (final) healing phase from the unspecific defending phenomenon at the leukocyte level.

The comparative analysis of the batches (fig. 2; 3) shows that the values of the leukocyte formula are in general higher for the batch 1DDW as compared with the batch 1DW with significant statistical differences. An exception makes the lymphocytes where an insignificant small decrease has been observed ($p=0.0000$). The analysis of the neutrophils mean values of the batches DW and DDW show insignificant differences ($p=0.4057$) a day after the cadmium chloride administration; but significant ones for the DDW ($p=0.0002$) after 62 days of treatment. This phenomenon suggests that in the case of the DDW batches the neutrophile fight phase is prolonged.

Table 3.

The statistical parameters for the leukocyte formula in rats treated with drinking water (DW) and deuterium-depleted water (DDW) in conditions of CdCl_2 (20 ppm /kg) intoxication

Batch	Parameters	Leukocyte Formula				
		<i>Neutrophils</i>	<i>Eosinophils</i>	<i>Basophils</i>	<i>Lymphocytes</i>	<i>Monocytes</i>
DW1	x	27.75	1.95	0.25	65.00	5.05
	Sx	0.79	0.24	0.08	1.02	0.41
	s	2.51	0.75	0.26	3.22	1.30
	CV	9.05	38.54	103.28	4.95	25.82
DW2	x	41.00	0.50	0.00	55.40	3.00
	Sx	0.58	0.12	0.00	1.68	0.51
	s	1.84	0.39	0.00	5.32	1.62
	CV	4.49	77.17	0.00	9.61	54.00
DW3	x	26.00	4.62	0.00	63.82	5.52
	Sx	0.79	0.14	0.00	0.35	0.19
	s	2.51	0.45	0.00	1.11	0.61
	CV	9.66	9.68	0.00	1.73	11.08
DDW1	x	35.05	3.45	0.00	55.05	6.45
	Sx	0.30	0.15	0.00	0.96	0.20
	s	0.94	0.46	0.00	3.03	0.63
	CV	2.68	13.29	0.00	5.50	9.76
DDW2	x	40.47	1.45	0.00	54.00	7.30
	Sx	0.77	0.16	0.00	0.47	0.11
	s	2.45	0.52	0.00	1.48	0.34
	CV	6.05	35.85	0.00	2.73	4.61
DDW3	x	48.10	4.45	0.00	40.90	6.55
	Sx	0.48	0.22	0.00	0.68	0.32
	s	1.52	0.69	0.00	2.15	1.00
	CV	3.17	15.39	0.00	5.26	15.21

x –mean; Sx- mean error of the average; Cv- variability coefficient

As concerning the mean value of the eosinophiles percentage it can be said that it has a similar evolution for both batches; following the physiological evolution of LF in the case of pathological stress. The batches 1 and 2 DDW show values that are significant higher ($p=0.0000$) what means that DDW had a preventive and stimulating effect of the early

reactivity that was significantly better than that of the DW. After 62 days the differences were insignificant ($p=0.5967$).

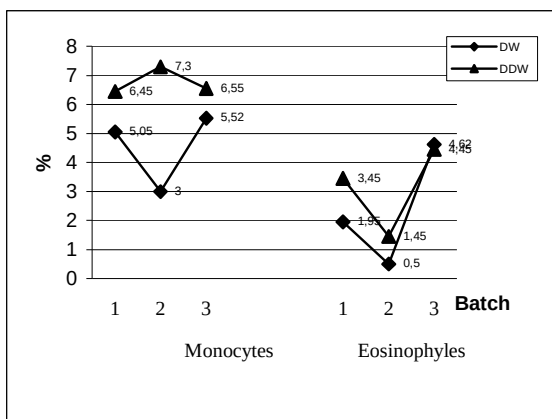


Fig. 2. The evolution of the monocytes and eosinophiles mean values in the rat batches treated with drinking water (DW) and deuterium-depleted water (DDW); intoxicated with cadmium chloride

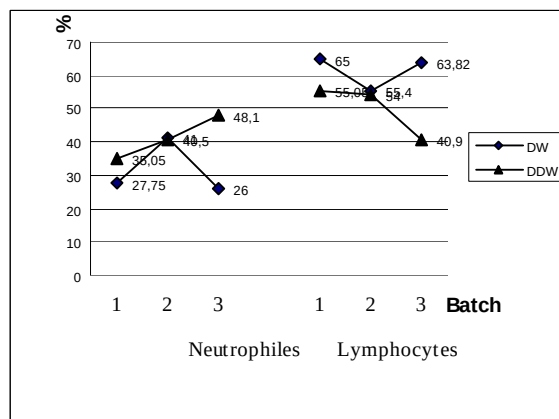


Fig. 3. The evolution of the neutrophils and lymphocytes mean values in the rat batches treated with drinking water (DW) and deuterium-depleted water (DDW); intoxicated with cadmium chloride

At the DDW batches the monocytes mean values were significantly higher; than those of the DW batches ($p=0.0002-0.0140$) thus suggesting that DDW stimulates the non-specific defence reaction of the organism both in normal and pathological conditions. At the DW batch; the monocytes evolution curve is that known from the specialized literature (8; 20) i.e. the monocytes' participation is accentuated only during the late phases of the leukocytes reaction.

The evolution of the lymphocytes mean values at the DDW batches shows a continuous decrease; as compared with the DW batches; where the decrease took place only after the administration of CdCl_2 and it was followed by a return towards the initial values. It can be said a day after the administration of the cadmium chloride the DW did not influence the specific defence; because $p=0.7055$ shows an insignificant difference between the lymphocytes values at the 2DW and 2DDW batches; but over a longer period of time (62 days) at the 3 DDW batch the difference was significantly reduced ($p=0.0002$)

From the point of view of the leukocytes formula modification; the usage of the DDW as a protective agent in the case of the cadmium chloride intoxication raised the non-specific defence; represented by the neutrophils and the monocytes while the administration of DW had no effect. The modifications evolution is described in the specialized literature as normal for the non-specific reaction at the nosogenous factors.

As concerning the healing effect; DDW generated the growth of the leukocyte ratio in favour of the cells with fagocytic capacity and antioxidative effect (neutrophils and monocytes) as compared with those having an immune role (lymphocytes). This suggests that the administration of the DDW can have a protective effect in cadmium chloride intoxication as compared with DW. But if cadmium chloride induces an oxidative stress (through lipidic peroxydation; diminishing of the reduced glutathione; katalase and of the glutathione peroxydase) mostly at the liver and the kidney level (15) but also at the sanguine one (12; 13); the growth of the non specific reactivity of the leukocytes could be the result of the organism necessity to counteract its oxidative effect.

CONCLUSIONS

- In the absence of the pathogenous factor; the usage of deuterium depleted water instead of drinking water modified significantly both the haematocrit and the leukocyte formula; i.e. the haematocrit decreased within the physiological limits and non-specific defence at the leukocyte level rose.
- As compared to drinking water; after the administration of a unique dose of cadmium chloride the deuterium depleted water modified significantly positive both the evolution of the haematocrit and the leukocyte formula; i.e. the haematocrit was stabilised and the non-specific defence of the leukocyte formula was stimulated early.
- From both the healing and preventing effect point of view as a result of the DDW administration an improvement of the non-specific defence capacity of the organism was produced. This can be a starting point for further studies that can establish the DDW status as adjuvant in the prevention and /or treatment of the different pathological states.

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